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Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development

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ABSTRACT

Few studies have been performed on changes to physicochemical and functional properties during caper fruit development. In this work, a comparative study on the evolution of physical, chemical, and nutritive parameters and bioactive compounds of three Spanish caper cultivars was performed for the first time. These fruits were characterized by presenting the exocarp green in all stages of development, with very slight changes, which were not produced by an increase in the synthesis of carotenoids, but by partial degradation of chlorophylls a and b. There was a decrease in the protein content of the caper fruits. The H-TAA was approximately twice as much as the L-TAA. The amounts of total phenols (TPC), total flavonoids (TFC), and total flavonol compounds (TFoC) were very high in all stages of fruit development, as approximately 60% of the total phenols (TPC) were total flavonoids (TFC) and 60% of the TFC were TFoC. Given that the caper is a common perennial xerophytic shrub with a remarkable adaptability to harsh environments, and given the excellent properties of these fruits, it could be considered a species of great interest for its cultivation due to its resilience against climate change.

1. Introduction

The weather is changing due to climate change and global warming. There is already evidence of this process in agriculture, and higher temperatures and more drought mean that agricultural production is being lost (Ray et al., 2015). High temperatures can cause damage to plant tissues as well as their metabolism and physiology, which can cause a decrease in the growth of plants (Ohama et al., 2017), and if the increase in temperature is excessive, it can lead to the death of plants and crop losses (Yamori et al., 2014). Given this situation, xerophilous plants can have great value because they can adapt to global warming. The caper (*Capparis spinosa* L.) is a common perennial xerophytic shrub with a remarkable adaptability to harsh environments (Chedraoui et al., 2017). This species is a plant that grows in dry and arid environments and it is found in Mediterranean regions in both cultivated and non-cultivated wildness. *C. spinosa* can change its leaf, stem, and root structures when adapting to drought conditions (Gan et al., 2013). The leaf, stem, and root of *C. spinosa* under drought conditions were better developed than those under normal conditions (Gan et al., 2013). These characteristics make the caper be considered as a colonizing plant, which in fact, can be found not only cultivated, but also wild among abandoned terraces and growing between rocks. Its distribution stretches from the Atlantic coast of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, in the Mediterranean basin and eastward to the Caspian Sea, and into Iran. It grows in North Africa, Europe, West Asia, Afghanistan, and Australia (Inocencio et al., 2006; Fici, 2014). This plant species is of great interest for its medicinal/pharmacological properties and its culinary uses. Its phytochemical importance relies on many bioactive components present in different organs and its cultivation can be of considerable economic value (Chedraoui et al., 2017). Its curative and medicinal properties have been known since ancient times and are linked to the presence of bioactive compounds of an antioxidant nature (flavonoids, flavonols), sugars, alkaloids, vitamins, etc. (Tlili et al., 2011). The plant has been used traditionally to prevent and/or treat a number of health disorders such as diabetes, hepatitis, obesity, and kidney problems (Anwar et al., 2016). Its anticarcinogenic potential (Kulisic-Bilusic et al., 2012), anti-arthritic, anti-inflammatory (Talat et al., 2015), and antibacterial effects (Nabavi et al., 2016) have also been proven. In addition, different parts of capers, like the fruits and caper buds, are usually pickled and added to salads, sauces, and jams (Anwar et al., 2016). Therefore, the caper is not only able to reduce erosion and slow down the desertification process, but can also do so productively, as a cost-effective alternative to other species.

This species has been cultivated since ancient times; however, in recent years its production has decreased significantly worldwide, with

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a decline in caper exports to testimonial values as of 2015 (UN Comtrade, 2017). In Spain, the production of capers buds decreased from 765 tons in 1999 to a production of 61 tons in 2009 (MAPAMA, 2017), mainly due to the cost of labor.

Therefore, the great potential of *C. spinosa* must be valued and its cultivation increased again, especially in areas such as the Mediterranean basin where soils are increasingly more arid, with unique crops of this species or together with other fruit species (Chedraoui et al., 2017), for which it is necessary to thoroughly understand this species. Many investigations have been conducted with caper buds, and several reviews have been carried out on the beneficial properties of this species for health; however, much less attention has been paid to the study of fruits that can be called caper fruits (Arrar et al., 2013), caper berries (Allaith, 2016; Jiménez-López et al., 2018), or caberberry (Legua et al., 2013). Therefore, the objective of this work was to study the physicochemical composition and antioxidant activity of caper fruits. As far as can be ascertained, no comprehensive studies on the development of caper fruits have been done. This is the first paper that studies the evolution of physicochemical parameters, antioxidant activity, and total phenols during three stages of caper fruit development.

2. Materials and methods

2.1. Plant material

Caper fruits of three cultivars, 'Orihuela 4' ('ORI 4'), 'Alberca 2' ('ALB 2'), and 'Alcayna 2' ('ALC 2') were hand-collected during two consecutive seasons, 2016 and 2017. 'ORI 4' was collected in Orihuela (Alicante, Spain), 'ALB 2' in La Alberca (Murcia, Spain) and 'ALC 2' in La Alcayna (Murcia, Spain). All cultivars were grown in rainfed plots and harvested in June, July, and September 2016 and 2017. The caper fruits were classified into three stages of development (Fig. 1): *finos*, with diameters less than 13 mm (thin); *medianos*, between 13–20 mm (medium); and *gruesos*, with diameters larger than 20 mm (thick) (BOE, 1984). These three stages of fruit development correspond to phenological stages 73, 75, and 79, respectively (Legua et al., 2013). One hundred fruits were harvested per stage and cultivar, and after performing non-destructive measures, they were stored the same day at −80 °C.

2.2. Physical parameters

The following physical parameters were measured in 30 fruits per stage and cultivar: equatorial diameter (mm) and fruit length (mm) using a digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK); fruit weight (g) was measured using a digital balance (model BL-600; Sartorius, Madrid, Spain). Instrumental color was on the surface of the caper fruits at two opposite points of the equatorial zone. Color was assessed according to the *Commission Internationale de l'Eclairage* (CIE*Lab*) and expressed as L^*, a^*, b^*, and Chrome, with a Minolta C-300 Chroma Meter spectrophotometer (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor.

2.3. Biochemical parameters

The following parameters were measured in triplicate, with 70 fruits destined to the analysis of biochemical parameters: (i) Chlorophylls a and b were extracted from each sample using 85% acetone (AOAC, 1990). Absorbance was read at 664 and 647 nm, using a Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). Results were expressed as mg × 100 g^{-1} fresh weight (fw); (ii) Total carotenoids were extracted according to Valero et al. (2011) with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoid content by reading the absorbance at 450 nm, and the results were expressed as mg of carotenoids × 100 g^{-1} fw, taking into account $\varepsilon_{cm}^{1\%} = 2560$; (iii). The protein content was analyzed by the Bradford (1976) method, using the Bio-Rad reactive and quantified according to Almansa et al. (2016). Results were expressed as mg × g^{-1} fw.

Extracts of caper fruits for the analysis of hydrophilic-total antioxidant activity (H-TAA) and lipophilic-total antioxidant activity (L-TAA) were prepared using Tris-acetate buffer pH 6.0, 20 mM $CaCl_2$, and ethyl acetate to separate the aqueous and organic phases, respectively (Arnao et al., 2001). The results were expressed as mg Trolox equivalent × 100 g^{-1} fw.

For the antioxidant activity determination by the ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) methods, a methanol extract was prepared as described by Wojdyło et al. (2013). The free radical scavenging capacities were determined by three methods, ABTS (Re et al., 1999), DPPH· radical (Brand-Williams et al., 1995), and FRAP (Benzie and Strain, 1996). Calibration curves, in the range of 0.5–5.0 mmol Trolox × L^{-1}, were prepared for the three methods and showed good linearity ($R^2 = 0.998$). Antioxidant capacity analyses were run in triplicate, and the results were expressed as mM Trolox fw.

Total phenolic compounds (TFC) were quantified in the hydrophilic phase according to Singleton et al. (1999) using the Folin–Ciocalteu reagent. A calibration curve was performed with gallic acid and the results were expressed as mg GAE x 100 g^{-1} fw.

Total flavonoid (TFC) and total flavonol (TFoC) compounds were extracted following the Zhuang method (1992) with 80% methanol. The analysis of total flavonoids was performed by spectrophotometry following the Zhuang method (1992) with $NaNO_2$, 10% $AlCl_3$, and 1 M

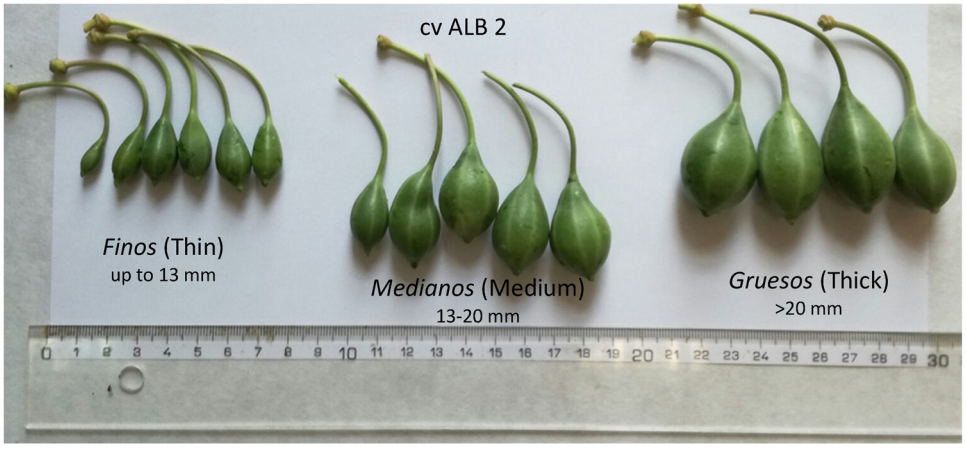

Fig. 1. Different sizes of caper fruits 'ALB 2' cultivar.

NaOH, and absorbance at 512 nm was measured on a Helios Gamma spectrophotometer (model UVG 1002E; Helios, Cambridge, UK). The results of total flavonoids were expressed in mg rutin equivalents x 100 g^{-1} fw. For this, a rutin calibration line was performed whose equation was y = 4.479x + 0.06773 with a correlation of 99.88%. Quantification of total flavonols was performed by spectrophotometry following the method of Kumaran et al. (2007) with $AlCl_3$ (2 mg mL^{-1}) and sodium acetate, and the absorbance at 440 nm was measured on a Helios Gamma spectrophotometer (model UVG 1002E; Helios, Cambridge, UK). Results of total flavonoids were expressed in mg rutin equivalents × 100 g^{-1} fw. For this, a rutin calibration line was performed whose equation was y = 3.408x + 0.0297 with a correlation of 99.01%.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS 18.0 software package for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test (ANOVA) for mean comparisons. The method used to discriminate among the means (multiple range test) was Fisher's LSD (least significant difference) procedure at a 95.0% confidence level.

3. Results and discussion

3.1. Dimensions of caper fruit

Data showed that there was a significant difference in size among the three stages of development in each cultivar (Table 1). The caper fruit weight of cultivar 'ORI 4' varied between 2.3 and 12.4 g, those of 'ALB 2' varied between 0.9 and 8.0 g, and those of 'ALC 2' varied between 1.4 and 10.2 g. This was expected since the three stages of development were classified by their diameter (BOE, 1984). However, it was observed that the caper fruits of 'ORI 4' were significantly larger than both 'ALB 2' and 'ALC 2', which were significantly light, in the three stages of development. These differences in the weight led to differences in the diameter and length, with cultivar 'ORI 4' (heavier fruit) having the longest length among than the cultivars. However, the diameter of 'ORI 4' was only greater than the other two cultivars in the thin stage, but not the medium or thick stages. This was due to the shape of the caper fruits of these three cultivars, since 'ORI 4' in its fully developed stage was elongated (50.4 mm), while 'ALB 2' and 'ALC 2' were almost rounded, only slightly elongated. This was visualized in the length/diameter ratio, which reached values higher than 2 in the caper fruits of 'ORI 4' in the thick stage (2.4), while it was significantly lower in the other cultivars with a value of 1.7 in 'ALB 2' and 'ALC 2' cultivars.

The fruit weight and dimensions might be influenced by several factors, such as cultivar genotype, weather conditions, ecology, and crop load (Gao et al., 2012).

3.2. Biochemical properties of caper fruit

Caper fruits were characterized by having an external exocarp green color in all stages of development, even its maturity and natural fruits opening. Only the mesocarp and endocarp can change color, from white to reddish or yellowish, when the fruit ripens. In this paper, we have analyzed the color of the fruit exocarp, so the caper fruits were green, with slight intensities of this color in the three stages of development studied (Table 2). Parameter a* quantifies the green color with negative values and parameter a* was negative in all stages in the three cultivars, between −14.0 to −8.4. The L* parameter did not show significant differences between the three stages of development studied in either in 'ORI 4' or 'ALB 2' (between 45.7 to 51.7), whereas 'ALC 2' showed greater luminosity when the stage of development increased (between 46.1 to 56.9). This increase in brightness was due to a more yellowish color when increasing development, as it was verified since parameter

Table 1
Morphology of caper fruits as affected by cultivar and stage of development.

Stage	Weight (g)			Diameter (mm)			Length (mm)			Length /Diameter		
	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'
Thin	2.3 ± 0.2 Ab[a]	0.9 ± 0.1 Aa	1.4 ± 0.2 Aa	11.4 ± 0.3 Ab	8.3 ± 0.6 Aa	10.5 ± 0.4 Ab	30.2 ± 1.2 Ab	19.1 ± 0.8 Aa	17.4 ± 0.8 Aa	2.6 ± 0.1 Ac	2.4 ± 0.1 Cb	1.7 ± 0.1 Aa
Medium	5.8 ± 0.4 Bb	4.3 ± 0.3 Ba	4.6 ± 0.5 Bab	16.4 ± 1.0 Ba	16.6 ± 0.5 Ba	16.7 ± 0.7 Ba	42.6 ± 0.8 Bc	30.8 ± 0.5 Bb	27.3 ± 1.0 Ba	2.7 ± 0.1 Ab	1.9 ± 0.0 Ba	1.6 ± 0.0 Aa
Thick	12.4 ± 1.1 Cb	8.0 ± 0.5 Ca	10.2 ± 0.9 Cab	20.8 ± 0.3 Ca	21.6 ± 0.5 Ca	21.9 ± 0.5 Ca	50.4 ± 2.2 Cb	36.0 ± 0.6 Ca	37.9 ± 1.3 Ca	2.4 ± 0.1 Ab	1.7 ± 0.0 Aa	1.7 ± 0.0 Aa

[a] Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant diff ;erence (LSD) procedure at 95% confidence level (n = 30). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 2
Colour of caper fruits as affected by cultivar and stage of development.

	L*			a*			b*			Chrome		
Stage	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'
Thin	46.7 ± 0.6 Aa[a]	53.1 ± 0.8 Ab	46.1 ± 1.0 Aa	−9.2 ± 0.2 Bb	−10.9 ± 0.1 Aa	−11.4 ± 0.8 Ba	18.4 ± 0.8 Ab	2.3 ± 1.1 Ac	14.8 ± 1.0 Aa	20.4 ± 0.8 Ab	27.19 ± 1.1 Ac	18.6 ± 1.2 Aa
Medium	45.7 ± 1.2 Aa	52.3 ± 0.9 Ab	53.3 ± 1.1 Bb	−8.4 ± 0.2 Cc	−10.4 ± 0.2 ABb	−13.2 ± 0.5 Aa	18.6 ± 0.4 Aa	25.1 ± 0.6 Ab	19.1 ± 1.0 Ba	20.3 ± 1.2 Aa	27.2 ± 0.9 Ac	23.2 ± 1.1 Bb
Thick	47.2 ± 1.2 Aa	51.7 ± 0.9 Ab	56.9 ± 1.2 Cc	−13.5 ± 0.6 Aa	−10.1 ± 0.2 Bb	−14.0 ± 0.4 Aa	20.2 ± 1.1 Aa	24.0 ± 0.6 Ab	22.1 ± 0.9 Cab	24.3 ± 1.2 Ba	26.0 ± 0.6 Aa	26.2 ± 0.9 Ba

[a] Values (means ± standard error) followed by the same letter were not significantly diff ;erent according to Fisher's least significant diff ;erence (LSD) procedure at 95% confidence level (n = 30). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 3
Photosynthetic pigments (mg × 100^{-1} g fw) of caper fruits as affected by cultivar and stage of development.

	Chlorophyll *a*			Chlorophyll *b*			Total chlorophyll			Carotenoids		
Stage	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'
Thin	11.0 ± 0.6 Ba[a]	15.2 ± 1.5 Aa	15.0 ± 2.0 Ba	5.2 ± 0.5 Ba	7.2 ± 1.0 Ba	6.7 ± 0.3 Ba	16.3 ± 1.1 Ba	22.5 ± 2.5 Aa	21.7 ± 2.3 Ba	1.1 ± 0.4 Aa	1.5 ± 0.0 Ba	1.7 ± 0.2 Aa
Medium	11.7 ± 1.1 Ba	15.4 ± 0.8 Aa	15.1 ± 1.9 Ba	5.6 ± 0.3 Ba	9.6 ± 0.0 Aba	7.4 ± 0.9 Bab	17.3 ± 1.4 Ba	25.0 ± 0.8 Aa	22.5 ± 2.1 Ba	1.5 ± 0.2 Aa	1.1 ± 0.0 Aa	1.3 ± 0.1 Aa
Thick	5.8 ± 1.0 Aa	11.6 ± 1.5 Ab	6.8 ± 1.0 Aa	3.8 ± 0.8 Aa	5.4 ± 0.7 Aab	2.8 ± 0.3 Aa	9.7 ± 1.7 Aa	17.0 ± 2.2 Ab	9.6 ± 1.3 Aa	1.2 ± 1.3 Aa	1.0 ± 0.0 Aa	1.2 ± 0.2 Aa

[a] Values (means ± standard error) followed by the same letter were not significantly diff ;erent according to Fisher's least significant diff ;erence (LSD) procedure at 95% confidence level (n = 3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

b*, which quantifies the yellow color, increased in 'ALC 2' (between 14.8 to 22.1) but not in 'ORI 4' or 'ALB 2' (between 18.4–30.7). Thus, the color intensity (Chrome index) increased in 'ALC 2' by increasing the stage of development (between 18.6 and 26.2) and 'ORI 4' (between 20.4 and 24.3), but not 'ALB 2'. The fruits modify their coloration due to synthesis and degradation of pigments throughout their development and maturation (Pék et al., 2010). In this case, the slight color changes were not produced by an increase in the synthesis of carotenoids (Table 3), since the carotenoid content was maintained in the caper fruits (between 1.1 to 1.7 mg carotenoids $\times 100^{-1}$ g fw), while there was a degradation of chlorophylls (a, b, and totals) by increasing the development of the caper fruits (between 15.4 and 5.8 mg chlorophyll $a \times 100^{-1}$ g fw). There were no significant differences between cultivars in either the carotenoid content or in total chlorophylls. The carotenoid contents were low compared to those obtained by Allaith (2016) with ripened caper fruits in Bahrain (3.9 mg $\times$ 100 g^{-1} fw).

The sugar and organic acid contents were analyzed by HPLC (data not shown); however, in all cases, the contents were below the detection limit of the device. However, Sher and Alyemeni (2010) found 0.4 g $\times$ 100 g^{-1} of sugar in caper fruits. Ren et al. (2012) found 6 organic acids in caper fruits, although they did not say at what concentration.

In the three cultivars, there was a decrease in the protein content of the caper fruits, with significant differences between the initial and final stages in all cases (Table 4). The protein content was higher in cultivars 'ALB 2' (between 1.1 to 0.7 mg protein $\times$ g^{-1} fw) with significant differences with cultivar 'ORI 4' (between 0.7 to 0.4 mg protein $\times$ g^{-1} fw). These protein contents were low compared to those obtained in mature caper fruits (2 g $\times$ 100 g^{-1} fw) by Sher and Alyemeni (2010).

3.3. Total antioxidant activity (TAA) of caper fruit

The total antioxidant activity was quantified in caper fruits by three methods: ABTS$^+$, DPPH·, and FRAP (Table 5). In addition, the total antioxidant activity was quantified by the ABTS$^+$ method in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions of the caper fruits (Table 4). In this table, it was observed that the H-TAA was maintained or increased parallel to the stage of development of the caper fruits, with values between 24.0 and 102.6 mg Trolox eq x 100^{-1} g fw. Cultivar 'ORI 4' maintained the H-TAA, with a final value of 53.1 mg Trolox eq $\times 100^{-1}$ g fw, but the H-TAA significantly increased in 'ALB 2' and 'ALC 2' with the stage of development of the fruit, with final values of 102.6 and 67.4 mg Trolox eq $\times 100^{-1}$ g fw, so 'ALB 2' was the cultivar that reached the highest H-TAA content, although it was only significant with respect to 'ORI 4'. L-TAA was approximately half that of H-TAA in the 'ORI 4' and 'ALC 2' cultivars in the three stages of fruit growth, with values ranging from 23.2 to 31.8 mg Trolox eq x 100^{-1} g fw. However, the L-TAA behavior was the same as H-TAA, that is, maintaining itself, as in the case of 'ORI 4' and 'ALC 2', or increasing parallel to the increase in the stage of development of the fruit ('ALB 2'). Therefore, the L-TAA was also higher in the cultivar 'ALB 2' and significantly lower in the other two cultivars, which did not show significant differences between them.

Antioxidant activity by the ABTS method (Table 5) was maintained ('ALB 2') or decreased ('ORI 4' and 'ALC 2') by increasing the stage of fruit development between 8.6 and 1.2 mM Trolox fw in the three cultivars. 'ALB 2' was the cultivar that had the highest antioxidant activity by the ABTS method in the most developed stage of the fruit. This antioxidant activity by the ABTS method was lower than those obtained by Allaith (2016) in mature caper fruits. Practically the same tendency was found when analyzing the antioxidant activity by the FRAP method, since antioxidant activity in 'ORI 4' and 'ALC 2' decreased significantly by increasing the stage of fruit development, while the behavior of the cultivar 'ALB 2' was the opposite, increasing the antioxidant activity with the development of the fruit. The values of the FRAP method oscillated between 0.3 and 12.5 mM Trolox fw. Allaith (2016) also found higher values than those found in this work in caper fruits from Bahrain. However, the antioxidant activity quantified by the DPPH method showed a different trend, with significant increases in the antioxidant activity of the caper fruits parallel to the increase in the fruit development with values ranging between 5.2 and 16.1 mM Trolox fw, being maximum in the 'ALB 2' cultivar and minimum in 'ALC 2' among which there were significant differences. These values were superior to those found by Allaith (2016) in caper fruits from Bahrain. Factors such as geographical source and genotype, but mainly maturity stage at harvest, may account for the observed divergence. The variation of the antioxidant capacity between cultivars could be due to differences in antioxidant compounds and their effectiveness (Robards et al., 1999).

3.4. Totals phenol, flavonoids, and flavonols of caper fruit

The cultivar factor significantly affected TPC in caper fruits (Table 6). 'ALB 2' was the cultivar that showed a significantly higher content than the other two cultivars studied. This cultivar showed a significant increase in TPC parallel to the development of the fruit, while the other two cultivars showed the same TPC content in the three stages of the fruit development studied. The TPC values ranged between 61.5 and 119.2 mg GAE x 100 g^{-1} fw. This maximum value was equal to that obtained for mature caper fruit from Bahrain by Allaith (2016) and from Spain by Jiménez-López et al. (2018), and higher than those found by Arrar et al. (2013). This study showed higher TPC contents in caper fruits as compared to other fruits, such as avocado (21.86 mg GAE x 100 g^{-1} fw), mango (3.037 mg GAE x 100 g^{-1} fw), and apple (58.12 GAE x 100 g^{-1} fw) (Fu et al., 2011). It is known that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested in the form of a diet rich in fruits and vegetables (Weisburger, 1992). Therefore, our results have demonstrated that caper fruits could be considered a good source of phenolic compounds and it would be recommended to consume caper fruits to ensure a maximum intake of phenolic compounds.

Arrar et al. (2013) found that 71.2% of the TPC in caper fruits were TFC, while Allaith (2016) found that it was 33%. We have also found that most TPC were TFC, about 60%, according to stage and cultivar (Table 6). The TFC values ranged between 39.5 and 81.6 mg eq rutin x 100 g^{-1} fw. The TFC content practically did not vary between stages of development or cultivars. Similar values were found by Jiménez-López

Table 4
Protein (mg $\times$ g^{-1} fw) and TAA in both hidro and liposoluble (mg Trolox x 100 g^{-1} fw) fractions of caper fruits as affected by cultivar and stage of development.

	Protein			H-TAA			L-TAA		
Stage	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'
Thin	0.7 ± 0.1 Ba	1.1 ± 0.0 Cb	1.1 ± 0.1 Bb	68.3 ± 4.1 Ac	24.0 ± 3.8 Aa	40.3 ± 6.0 Ab	23.9 ± 4.2 Aa	44.7 ± 3.1 Ab	23.2 ± 4.3 Aa
Medium	0.5 ± 0.6 ABa	0.8 ± 0.0 Ba	1.0 ± 0.0 Bb	54.1 ± 13.2 Ab	76.7 ± 9.3 Bb	43.4 ± 6.4 Aa	23.9 ± 2.4 Aa	67.2 ± 6.5 Bb	22.1 ± 2.3 Aa
Thick	0.4 ± 0.0 Aa	0.7 ± 0.0 Ab	0.7 ± 0.0 Ab	53.1 ± 2.2 Aa	102.6 ± 17.8 Bb	67.4 ± 0.5 Bab	27.5 ± 2.8 Aa	75.3 ± 2.6 Bb	31.8 ± 3.3 Aa

[†]Values (means ± standard error) followed by the same letter were not significantly diff ;erent according to Fisher's least significant diff ;erence (LSD) procedure at 95% confidence level (n = 3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 5
Antioxidant activity (mM Trolox fw) of caper fruits as affected by cultivar and stage of development.

	ABTS			DPPH			FRAP		
Stage	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'
Thin	8.6 ± 0.5 Ba	7.6 ± 1.1 Aa	8.1 ± 0.5 Ba	5.2 ± 0.2 Aa	5.5 ± 0.4 Aa	6.0 ± 0.1 Aa	6.7 ± 0.9 Ba	6.1 ± 0.8 Aa	7.4 ± 0.2 Ba
Medium	1.1 ± 0.1 Aa	7.6 ± 0.1 Ac	3.3 ± 0.6 Ab	14.9 ± 0.6 Bab	15.5 ± 0.9 Bb	12.5 ± 0.9 Ba	0.3 ± 0.0 Aa	11.8 ± 0.2 Bc	3.0 ± 0.4 Ab
Thick	1.2 ± 0.0 Aa	8.5 ± 0.6 Ab	1.9 ± 0.2 Aa	14.5 ± 0.4 Bab	16.1 ± 1.3 Bb	13.3 ± 0.2 Ba	0.3 ± 0.9 Aa	12.5 ± 0.7 Bc	3.0 ± 0.4 Ab

[†]Values (means ± standard error) followed by the same letter were not significantly diff ;erent according to Fisher's least significant diff ;erence (LSD) procedure at 95% confidence level (n = 3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 6
Total phenols, flavonoids and flavonols contents of caper fruits as affected by cultivar and stage of development.

	Total phenols (mg GAE × 100 g^{-1} fw)			Total flavonoids (mg eq. rutin × 100 g^{-1} fw)			Total flavonols (mg eq. rutin × 100 g^{-1} fw)		
Stage	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'	'ORI 4'	'ALB 2'	'ALC 2'
Thin	61.5 ± 2.5 Aa[a]	74.6 ± 3.5 Aab	86.2 ± 9.6 Ab	44.5 ± 3.6 Aa	51.2 ± 3.3 Aa	39.5 ± 0.4 Aa	31.3 ± 2.5 Aa	39.9 ± 0.8 Aa	39.7 ± 4.1 Ba
Medium	67.8 ± 3.3 Aa	101.3 ± 3.4 Bb	77.8 ± 5.5 Aa	52.9 ± 1.7 Aa	81.6 ± 5.7 Bb	41.2 ± 4.7 Aa	30.9 ± 3.1 Aa	36.0 ± 3.7 Aa	28.4 ± 3.1 Aa
Thick	66.8 ± 4.9 Aa	119.2 ± 0.6 Cb	73.4 ± 7.0 Aa	53.1 ± 7.1 Aa	66.2 ± 10.7 ABa	47.6 ± 9.8 Aa	30.9 ± 4.4 Aab	36.1 ± 2.2 Ab	22.2 ± 1.2 Aa

[a] Values (means ± standard error) followed by the same letter were not significantly diff ;erent according to Fisher's least significant diff ;erence (LSD) procedure at 95% confidence level (n = 3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

et al. (2018) in ripened caper berries. The TFC content in caper fruits was very high and many phenolic compounds were found in this fruit, such as kaempferol-3 rutinoside, quercetin-3-*O* rutinoside (rutin), sakuranetin, isoginkgetin, ginkgetin (Musallam et al., 2012; Zhou et al., 2011), epicatechin, and myricetin (Francesca et al., 2016), among others. The flavonoids found in caper fruits were their main pharmacologically active compounds, and were linked with antioxidant and anticarcinogenic effects (Anwar et al., 2016). In addition, caper fruits have traditional medicinal applications, including diuretic, expectorant, and astringent activities and treatment of tuberculosis, atherosclerosis, hepatitis, and kidney diseases. The fruit also acts as a tonic, and is used to expel worms from the intestine and gas from the stomach (Anwar et al., 2016).

Arrar et al. (2013) found that 65% of the TFC in caper fruits are TFoC. We have also found that most TFCs are TFoC, with percentages of about 60%, according to stage and cultivar (Table 6). The TFoC values ranged between 22.2 and 39.9 mg eq rutin x 100 g^{-1} fw. The TFoC content practically did not vary between stages or cultivars. Most TFoC in caper fruits were rutin, quercetin, and myricetin (Francesca et al., 2016) and were responsible, among other compounds, for the healthy effects of the caper fruits (Anwar et al., 2016).

4. Conclusion

Here, for the first time, we have presented a study including the changes that occur during the development of caper fruits. The study was carried out in three Spanish caper cultivars from three different geographical zones. The present data indicated that these fruits were characterized by presenting the exocarp green in all stages of development, with very slight changes that were not produced by an increase in the synthesis of carotenoids, but rather by partial degradation of chlorophylls a and b. We have also demonstrated that caper fruits have good antioxidant potential capacity. This effect may be attributed to the contents of total phenols, flavonoids, and flavanols. These effects could justify the wide use of this plant as food and in traditional medicine, and as a plant to obtain bioactive compounds for the food industry, while it can be planted in increasingly dry places such as the Mediterranean basin given the threat of global warming. The cultivar 'ALB 2' was the best of the three studied, since it was the one with the highest TAA content and total phenols and flavonoids. The thick stage of development is better to be eaten since it presented a higher content of bioactive compounds.

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